

Study Outline for the Toxicology Branch of the Columbia Environmental Research Center (CERC), USGS, Columbia MO

Study code: 13-20-03
Title: An evaluation of chronic (28- and 90- day) toxicity of NaCl to fatmucket (*Lampsilis siliquoidea*) in water-only exposures with or without silica sand

Lead Investigator: Ning Wang
Lead Technician: James Kunz
Date: December 12, 2012

CERC project number: 10-TOX-02 (EPA Region 5)
Basis+ project number: NA00EJR (EPA Region 5)
Basis+ task number: 20 (EPA Region 5)
USGS program element: 42100 (EPA Region 5)

Reviewed by: Chris Ingersoll

Juvenile mussels are sensitive to NaCl in acute exposures and the EPA acute water quality criterion may not adequately protect the mussels tested (Wang et al. 2012). It is unknown about chronic effect of NaCl on mussels. The objective of this study is to evaluate chronic NaCl toxicity to fatmucket in a 28-d water-only exposure following standard methods (ASTM 2012). A side-by-side longer-term (90-d) NaCl exposure will be also conducted to compare the mussel responses in the 28-d and 90-d exposures. In addition, to further evaluate the influence of sand (as a substrate) on mussel survival and growth in chronic exposures, the 28-d and 90-d exposures were conducted in test chambers with or without addition of fine sand.

Test conditions are summarized in Table 1. Juvenile mussels will be obtained from laboratory cultures at the CERC (fatmucket, about 2-month old and 1.5 mm shell length). The juvenile mussels will be acclimated to test water and temperature (20°C) for at least 48 h before starting tests. The toxicity tests will be conducted in two intermittent flow-through diluters. Stock solution of NaCl (99.9% purity; Sigma-Aldrich, Saint Louis, MO, USA) will be delivered with each cycle of the diluter by a Hamilton syringe pump (Hamilton, Reno, NV, USA). Each diluter delivered five exposure concentrations with a dilution factor of 0.5 plus a control. The nominal concentrations of NaCl will be 0, 62.5, 125, 250, 500, and 1000 mg NaCl/L. Eight 300-ml replicate glass beakers per concentration will be placed in each of the two temperature-controlled diluters at 20°C (four replicates with fine silica sand and another four replicates without any sand). Silica sand (Granusil #4030, Unimin Corporation) will be sieved to 500-µm particles and about 5 ml of sand will be added into each test beaker for the exposures with sand. Each beaker had a 2.5-cm hole in the side covered with 50-mesh (279-µm width opening) stainless-steel screen and contained 200 ml of water. The diluter provides 120 ml of test solution to each replicate beaker every 4 h (3.6 water volume additions per day). Ambient laboratory light (~500 lux) will be used with 16:8 h light:dark photoperiod.

At the start of the exposure, ten mussels exhibiting foot movement will be impartially transferred into each of four replicate beakers per exposure concentration per species. In addition, about 20 juveniles will be sampled and preserved in 8% formalin for initial length and weight measurement. The mussels will be fed twice daily with 2 ml of an algal mixture (*Nannochloropsis* concentrate and Shellfish Diet, Reed Mariculture, Campbell, CA, USA; Wang et al. 2007). The survival (foot movement within 5 min) of mussels in water-only beakers will be determined once every two weeks, and the survival of mussels in sand beakers will be determined once every four weeks. Surviving mussels on about Day 30 and Day 60 in the 90-d exposures will be transferred into new beakers (and new beakers containing new sand for the exposures with sand).

Water temperature will be monitored daily. Water quality (dissolved oxygen, pH, conductivity, hardness, and alkalinity) will be determined in the control, medium, and high concentrations every other week. Water conductivity will be measured at least three times per week to monitor the exposure concentrations. Composite water samples for the analysis of major anions (e.g., chloride and sulfate) will be collected from each of 6 exposure concentrations on Days 0, 14, and 28 during the 28-d exposures, and also on Days 0, 14, 28, 60, and 90 during the 90-d exposures.

At the end of the 28-d or 90-d exposures, survival of mussels will be determined. Live mussels in each replicate will be counted. Surviving mussels will be preserved in 8% formalin for subsequent shell length and dry weight measurement (mussels in each replicate dried at 60°C for 24 h and weighted in group). The acceptability criterion will be $\geq 80\%$ control survival for 28-d exposure (ASTM, 2012). There is no test acceptability criterion for survival in a longer-term 90-d exposure with juvenile mussels. We expect to have a control survival of $\geq 64\%$ for the 90-d exposure.

Effect concentrations (e.g., NOEC, LOEC, EC10, and EC20) for survival, growth (dry weight), and biomass (total dry weight of surviving organisms per replicate) will be determined using Toxicity Relationship Analysis Program (TRAP) obtained from Russell Erickson, USEPA, Duluth MN and TOXSTAT software (version 3.5, WEST 1996).

References

- American Society for Testing and Materials International. 2012. Standard guide for conducting laboratory toxicity tests with freshwater mussels (ASTM E2455-06). *Annual Book of ASTM Standards* Volume 11.06. West Conshohocken, PA.
- Wang N, Ingersoll CG, Greer IE, Hardesty DK, Ivey CD, Kunz JL, Brumbaugh WG, Dwyer FJ, Roberts AD, Augspurger T, Kane CM, Neves RJ, Barnhart MC. 2007. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environ Toxicol Chem* 26:2048-2056.
- Wang N, Ingersoll CG, Ivey CD, Hammer E, Bauer CR, Augspurger T, Raimondo S, Shephard B, Bartoszek J, Barnhart MC, Eckert N. Acute sensitivity of freshwater mussels to selected chemicals with differing toxic modes of action. Poster presented at the SETAC meeting, Nov. 15, 2012, Long Beach, CA.
- Western EcoSystems Technology. 1996. TOXSTAT® 3.5. Cheyenne, WY, USA.

Table 1. Summary of test conditions for conducting chronic NaCl toxicity test with fatmucket (*Lampsilis siliquoidea*) in basic accordance with ASTM (2012).

Parameter	Conditions
Test chemical:	NaCl
Test type:	Flow-through, water-only exposure with or without sand
Exposure duration:	28 or 90 days
Temperature:	20°C
Light quality:	Ambient laboratory light
Light intensity:	About 500 lux
Photoperiod:	16L:8D
Test chamber size:	300-ml glass beaker
Test solution volume:	Water-only treatment: 200-ml water Water + sand treatment: 5-ml sand and 195-ml water
Renewal of solution:	125 ml of additional water once every 4 hours
Age of test organism:	Fatmucket: about 2-months after transformation
Organisms/test chamber:	10
Replicates/concentration:	4
Feeding:	2-ml algal mixture twice daily (Wang et al. 2007)
Cleaning:	Surviving mussels on about Day 30 and Day 60 in the 90-d exposures will be transferred into new beakers (and new beakers containing new sand for the exposures with sand)
Dilution water:	Well water diluted with deionized water (about 100 mg/L hardness as CaCO ₃ , pH 8.3)
Dilution factor:	0.5
Test concentrations:	0, 62.5, 125, 250, 500, 1000 mg NaCl/L
Chemical residues:	Water samples for NaCl analysis will be collected from the 6 exposure treatments biweekly during the 28-d exposure and monthly during the 90-d exposure.
Water quality:	Dissolved oxygen, pH, conductivity, hardness, alkalinity, and ammonia will be measured in the control, medium, and high exposure concentrations biweekly. In addition, conductivity will be measured at least 3 times per week. Major anions (sulfate and chloride) will be measured in each exposure concentrations on Days 0, 14, and 28 during the 28-d exposure, and Days 0, 14, 28, 60, and 90 during the 90-d exposure
Endpoint:	Survival, shell length, dry weight, and biomass
Test acceptability:	≥80% control survival for 28-d exposure. No test acceptability criterion for 90-d exposure

Appendix 1. Waterbath request form.

1. Investigator/technician:	Ning Wang/James Kunz
2. Study code:	13-20-03
3. Date of request:	December 13, 2012
4. Chemical(s) or Treatment(s):	See Table 1.
5. Test organism(s):	See Table 1
6. Test organism culture:	See Table 1.
7. Temperature (°C):	20
8. Proposed Wet Lab:	RAS
9. Number of small diluters:	1
10. Number of large diluters:	0
11. Number of Hamilton pumps:	1
12. Number, type, size of chambers:	300-ml water-only glass beakers (8 replicates x 6 treatments x 2 test durations =96)
13. Number and type water splitter:	12
14. Water type:	Diluted well water (100 mg/L hardness as CaCO ₃)
15. Daily volume water use:	About 125 L
16. Effluent discharge:	Red Line (City)
17. Start date (including set up):	Late December 2012
18. Exposure duration:	28 or 90 days
19. End date (after cleaning and rehab to original condition):	Late March 2013
20. Flexibility in start date:	None
21. Special needs:	None
22. Response from Ingersoll:	Diluters #1 and #2 in the RAS wet lab were assigned for this project (CGI December 12, 2012)